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Vitamin Supplement

Increase of vitamin D_2 by UV-B exposure during the growth phase of white button mushroom (*Agaricus bisporus*)

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Abstract

Background: Mushrooms are the only non-animal food source of vitamin D. Wild mushrooms have naturally high vitamin D_2 content, and cultivated mushrooms produce vitamin D_2 from ergosterol when exposed to supplementary UV-B during the post-harvest phase.

Objectives: This study investigated the effects of providing supplementary UV-B during the growth phase on vitamin D_2 formation and the interactions with growth of mushrooms, as compared to supplementary UV-B during the post-harvest phase or exposure to sunlight for both cultivated and wild mushrooms.

Methods: Experiments were carried out with exposure to supplementary UV-B just prior to harvest in the range of 0–2,400 mJ cm^{-2}. Mushrooms grew for 2 days with or without repeated UV-B exposure each day. Vitamin D_2 and growth rate were determined. Some mushrooms were post-harvest treated by exposure at 200 mJ cm^{-2} supplementary UV-B or natural sunlight, prior to vitamin D_2 determination.

Results: The content of vitamin D_2 was 0.2–164 µg 100 g^{-1} fresh weight (FW), and there was a linear relationship between UV-dose up to 1,000 mJ cm^{-2} and vitamin D_2 content. The fast growth rate of the mushrooms diluted the vitamin D_2 from 24 to 3 µg 100 g^{-1} within 2 days of exposure at 200 mJ cm^{-2}. Following repeated UV-B exposure, vitamin D_2 increased to 33 µg vitamin D_2 100 g^{-1}. Growth was unaffected by UV-B. Post-harvest exposure to supplementary UV-B resulted in a higher vitamin D_2 content of 32 µg 100 g^{-1} compared to the 24 µg 100 g^{-1} obtained from exposure to UV-B during the growth phase. In contrast, wild and cultivated mushrooms with and without exposure to sunlight had vitamin D_2 content in the range of 0.2–1.5 µg vitamin D_2 100 g^{-1}.

Conclusions: This study showed that mushrooms with a well-defined content of vitamin D_2 can be obtained by exposure to supplementary UV-B just prior to harvest.

Keywords: *ergocalciferol*; *growth phase*; *post-harvest*; *sunlight*; *wild mushrooms*; *yield*

During winter, food is the main source of vitamin D, and fish and other animal products are considered important sources of vitamin D to fulfil daily requirement. Wild mushrooms have been recognised as the only non-animal food source of vitamin D with reports of high vitamin D_2 contents of 3–59 µg 100 g^{-1} fresh weight in, for example, *Cantharellus* sp. and *Boletus* sp. (1, 2). The content of vitamin D in wild relatives of the cultivated button mushroom, *Agaricus bisporus*, has not yet been reported.

Vitamin D_2 is formed during UV exposure, as a result of photochemical cleavage of the B ring of ergosterol forming the intermediate pre-vitamin D_2, this then undergoes thermal re-arrangement to ergocalciferol (vitamin D_2) (3). Several recent studies have reported the same response in mushrooms exposed to supplementary UV post-harvest. They showed that exposure to UV-B (280–315 nm) was more effective in the formation of vitamin D_2 than was exposure to UV-A or UV-C (4, 5). A linear relationship between the UV dose and the vitamin D_2 formed was found, and vitamin D_2 in the mushrooms was found to be relatively stable during post-harvest storage (6). Based on this knowledge, commercial production of button mushrooms with a high content of vitamin D_2 was established based on post-harvest exposure to UV. Cultivated mushrooms are marketed as a fresh and natural product; therefore, to underpin this product profile, the UV treatment could be integrated into the growth phase.

Such treatment to naturally enhance vitamin D_2 in mushrooms prior to harvest would be more representative of the processes occurring in wild mushrooms exposed to sunlight. To relate supplementary UV exposure in mushrooms to natural conditions, more knowledge is needed on the interactions between mushroom growth, UV exposure, and vitamin D_2 as well as on the effect of natural sunlight on vitamin D_2 content in cultivated and wild mushrooms.

The objectives of this study were to investigate the effects of (1) one or repeated exposures of supplementary UV-B prior to harvest on vitamin D_2 formation as compared to post-harvest exposure, (2) mushroom growth rate on vitamin D_2 content and of UV exposure on growth rate and (3) exposure of cultivated mushrooms to sunlight as compared to wild mushrooms on vitamin D_2 content.

Materials and methods

Two experiments were carried out with exposure of mushrooms to UV-B doses in the range of 0–250 mJ cm^{-2} over three days (Experiment A), and exposure of mushrooms to UV-B doses of 220–2,400 mJ cm^{-2} during one day (Experiment B).

The UV-B equipment

A transportable and adjustable UV-B unit was constructed using six UV-B tubes (Medical Therapy/12, Philips, Eindhoven, The Netherlands) giving a homogeneous distribution of UV-B over an area of 0.6×0.4 m^2. The intensity of UV-B was 1.88 mW cm^{-2} at a distance of 0.25 m between the mushrooms and the UV-B tubes. Exposure to UV-B for 1 min 46 sec provided a dose of 200 mJ cm^{-2}. The spectral distribution of the UV-B unit was measured by a spectrometer (AvaSpec-2048, Avantes, Eerbeek, The Netherlands) that was calibrated with a standard of spectral irradiance OL FEL-C (Optronic Laboratories, Orlando, FA, USA), a lamp that includes wavelengths down to 250 nm. The spectral composition was measured at 0.25 m distance from the UV-B tubes, with a black screen surrounding the unit to avoid stray light. Measurements were an average of 10 scans (Fig. 1). The UV-B doses in the different treatments were measured by the accumulated intensity over time by use of a UV-B photometer (IL1400 SEL005/TLS312/Td; International Light Technologies, MA, USA).

Experimental design

White button mushrooms (*Agaricus bisporus* (Lange) Imbach) were grown at 18°C day and night temperature and 88% relative humidity on horse manure/barley straw compost at the commercial production facility of Egehøj Champignon A/S, Veflinge, Denmark in 2009. Experiment A was initiated on the second sampling day of the second flush (7-day harvest cycle), 36 days after inoculation of the compost with mycelium. Experiment B was carried out on the first day of the third flush, 45 days after inoculation. In both experiments, the mushrooms were sampled for yield and vitamin D_2 analyses at harvest, according to the sampling practice for commercial harvest-ready mushrooms.

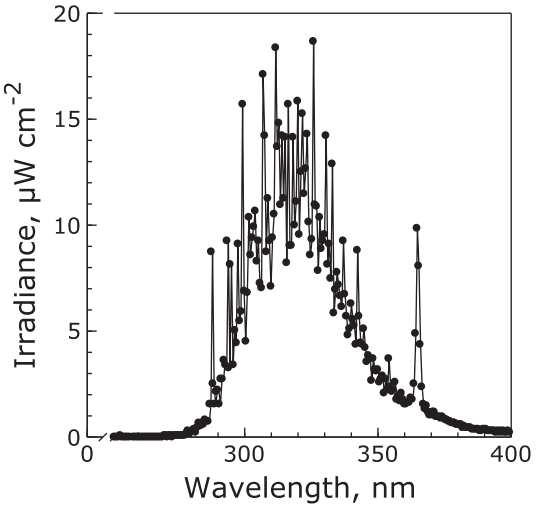

Fig. 1. The spectral distribution of the UV-B unit used for treatments of supplementary UV-B during the growth phase and post-harvest. The data are a mean of 10 scans and presented with 1.2 nm resolution.

In Experiment A, 28 plots of 0.4×0.4 m^2 were established on the shelves with growing mushrooms in a complete randomised statistical design with four blocks, two in each of two growth chambers giving four replicate plots per treatment. By varying the duration of exposure, the treatments were 0, 100, 150, 200 and 250 mJ m^{-2} supplementary UV-B on the first day; and 200 mJ cm^{-2} given repeatedly once a day on the first, second and third day of the experiment (3×200 mJ cm^{-2}). All plots were sampled immediately after exposure and also on the three consecutive days (at 24 hour periods) after the first day exposure to 0 or 200 mJ cm^{-2} and after the repeated exposure to 200 mJ cm^{-2}. Additional samples were taken for post-harvest treatments of exposure to 200 mJ cm^{-2} natural UV-B in sunlight for approximately 20 min at mid day, or following overnight cooling (2°C), re-adjustment to 18°C and exposure to 200 mJ cm^{-2} supplementary UV-B. Additional production areas from the four blocks were sampled the three days for estimation of biomass of small mushrooms, that remained after harvest to grow for next day harvest. This was to calculate vitamin D_2 recovery during the experiment.

In Experiment B, seven plots of 0.6×0.4 m^2 were established on the shelves with growing mushrooms in a growth chamber. Treatments at 220, 500, 1,000 and 2,400 mJ m^{-2} supplementary UV-B were given to the plots in a complete randomised statistical design with no replications in the range of 220–1,000 mJ m^{-2} and six

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replications of the 2,400 mJ m^{-2} treatment. All plots were sampled immediately after exposure.

In the period of late July to October 2009, four composite samples of each 2–3 wild growing mushrooms (*Agaricus sp.*) were sampled in an orchard at Aarslev, Denmark (10°27′E, 55°18′N) for determination of vitamin D_2 content. In addition, four samples of *A. bisporus* of miscellaneous origin (strains and producers) were purchased for determination of vitamin D_2 content.

All fresh mushroom samples were weighed, cooled (1°C, 100% relative humidity) and mushrooms counted, cleaned and base removed. Each sample of at least 200 g material was frozen at −18°C for subsequent freeze drying for determination of dry matter and vitamin D_2 content.

Vitamin D_2 analysis

Prior to analysis, the frozen samples of mushrooms were thawed to room temperature, homogenised for 30 sec (Type 320, Moulinex, Group SEB, F) and freeze-dried (Christ Beta 1–8, Martin Christ Gefriertrochnungsanlagen GnbH, Osterode, D). To achieve a homogenised subsample, the freeze-dried mushrooms were homogenised again for 30 sec (Type 320, Moulinex, Group SEB, F).

The analytical method and the equipment used to determine vitamin D_2 in the mushrooms were a modification of a method used for meat (7). In short, 1 g freeze-dried mushrooms were taken for analysis, and vitamin D_3 was added as an internal standard according to EN12821. The samples were extracted by alkaline saponification followed by clean-up by silica solid phase extraction. Due to the extremely high amount of ergosterol in the samples, another preparative HPLC system was used than in meat (7). The combination of a silica and an amino column was replaced by a shorter silica column (Luna, Si 60, 3 µm, 150 × 4.6 mm, Phenomenex, Torrance, CA, USA) in combination with an isocratic mobile phase consisting of 2-propanol:methyl-*tert*-butyl-ether:cyclohexan:*n*-heptan (0.7:2:48.65:48.65). A flow rate of 1.2 ml min^{-1} gave a retention time for vitamin D_2 of 11.2 min; sample collection was made during the interval 10.4–12.2 min. The retention time for ergosterol was 16.0 min, which was baseline separated from vitamin D_2. For separation, a C18 column combined with a mobile phase consisting of acetonitrile:methanol (80:20) was used, whereas detection was performed by photodiode array detector (220–320 nm) and quantification at 265 nm. The analysis was performed as single determination. For quality control, a house-reference material consisting of freeze-dried mushrooms was analysed on each day of analyses, showing an internal reproducibility of 5.8%. The analysis was run in a laboratory accredited after ISO 17025 for the original analytical method, and the correctness for the method was controlled by participation in the Food Analysis Performance Assessment Scheme (www.fapas.com).

Data and statistical analysis

Recovery of vitamin D_2 produced after the first day exposure to UV-B was calculated per unit production area over the three days of experiment based on the biomass of large harvested and small remaining mushrooms. For this, it was assumed that vitamin D_2 content in small mushrooms equalled that was measured in harvested mushrooms.

The data were subjected to analysis of variance. Multiple comparisons were based on values of the least significant difference derived from analysis of variance (Proc GLM, SAS Institute Inc., Cary, NC, USA). When appropriate, the results were transformed before analysis to obtain homogeneity of variance. Differences determined at $P < 0.05$ were considered statistically significant.

Results and discussion

Results from Experiments A and B showed a close to linear relationship between dose of UV-B and formation of vitamin D_2 at 0–1,000 mJ cm^{-2} supplementary UV-B during the growth phase. The outputs from the linear regressions were very similar with a-factors of 0.118 and 0.108 for the range of 0–250 mJ cm^{-2} and 0–1,000 mJ cm^{-2}, respectively, and high correlation coefficients (Fig. 2A and B). The linearity of the relationship found in these two experiments was previously reported by Roberts et al. 2008 (6) following post-harvest exposure in the range of 500–1,000 mJ cm^{-2} supplementary UV-B after overnight cooling of white button mushrooms. However, in the present study, the a-factor of the linear regression was 0.11 at exposure during the growth phase, compared to 0.06 calculated from Roberts et al. (2008) (6). Thus, exposure, for example, to 500 mJ cm^{-2} during the growth phase formed vitamin D_2 of approximately 57 µg 100 g^{-1} fresh weight compared to 37 µg 100 g^{-1} at post-harvest exposure (6). However, the difference between the two studies cannot be ascribed to a higher efficiency of vitamin D_2 conversion at exposure during the growth phase. In the present study, mushrooms sampled after overnight cooling and post-harvest exposure were found to have a higher vitamin D_2 content compared to those exposed during the growth phase, and this difference was not caused by effects of overnight cooling on dry matter content (Table 1). The higher conversion of vitamin D_2 in the present study compared with that of Roberts et al. (2008) (6) was probably caused by differences in spectral distribution of the UV-B from different types of lamps. Also, experimental conditions, such as temperature and moisture content, have been shown to affect the formation of vitamin D_2 (5). Orientation of the mushrooms to the UV source has also been a factor that contributes to vitamin D_2 level,

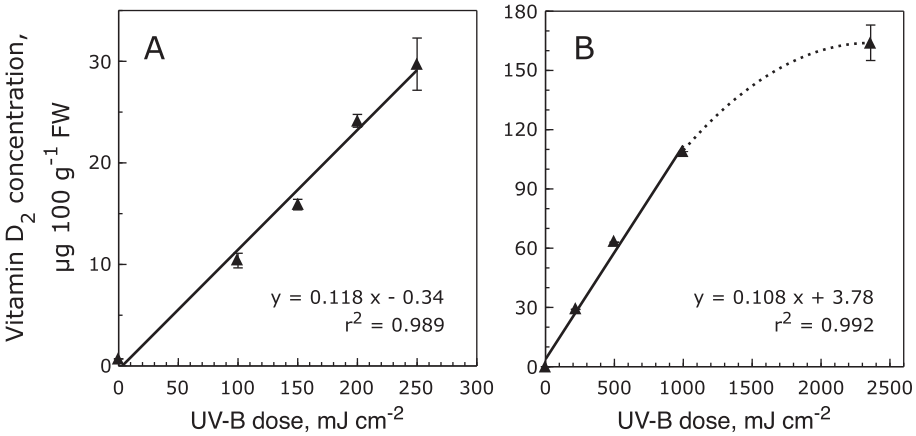

Fig. 2. The relationship between vitamin D_2 concentration in mushrooms and exposure to UV-B dose up to 250 mJ cm^{-2} in Experiment A and up to 2,400 mJ cm^{-2} in Experiment B. Error bars indicate standard errors in Experiment A ($n = 4$), and in Experiment B for the highest dose ($n = 6$). The equations are linear regression up to 250 and 1,000 mJ cm^{-2}. FW = fresh weight.

that is, the content of vitamin D_2 varies within an individual mushroom (8); however, in this study and that of Roberts et al. (2008) (6), the orientations of the mushrooms were similar. Finally, also the difference between our HPLC-DAD and the LC-MS/MS methods used in (6) may give a bias in the results.

The formation of vitamin D_2 levelled off at a content of 164 µg 100 g^{-1} at the high dose of 2,400 mJ cm^{-2} supplementary UV-B as also reported for doses beyond 1,200 mJ cm^{-2} by others (5, 6). In this study, a clear browning effect was observed on the surface of the exposed mushrooms indicating that at high doses of UV-B, there was damage to the surface cells (8). It has been suggested that high doses of UV cause photodegradation of vitamin D_2 (3). Thus, very high doses of UV-B are relatively less efficient for conversion of ergosterol to vitamin D_2 and cause surface discolouration.

UV-B exposure did not affect the growth rate of the mushrooms in Experiment A (Fig. 3). The average yields were 2.25, 4.83 and 3.36 kg m^{-2}, and the number of mushrooms were 236, 322 and 163 pieces m^{-2} on the first, second and third day of the flush, respectively. The dry matter contents were 8.4, 8.3 and 9.3 g 100 g^{-1}, respectively. Over the three days, the vitamin D_2 content of mushrooms decreased sharply from 24 to 3 µg 100 g^{-1} when measured after one exposure at 200 mJ cm^{-2} of supplementary UV-B (Fig. 4). This decrease was observed mainly from the first to the second day of the experiment. By Day 3, 94% of the vitamin D_2 formed during one exposure to UV-B on Day 1 could be accounted for in the large harvested and the small remaining mushrooms (Table 2). Thus, the fast growth rate of the mushrooms diluted the vitamin D_2 formed after the exposure on Day 1, as the high recovery of vitamin D_2 indicated that there was no significant degradation or transport of vitamin D_2 from the mushrooms.

When the UV-B exposure was repeated at daily intervals (Day 1–3), the vitamin D_2 content increased to 33 µg 100 g^{-1}. These results suggest that repeated exposure to UV-B is needed just prior to harvest and during all days of a flush to ensure a high level of vitamin D_2 in the final product. The increase in the vitamin D_2 content after repeated exposure may be explained by a carryover of vitamin D_2 formed in the small mushrooms left growing for the next day. There was no indication that one exposure to UV-B stimulated or inhibited the formation of vitamin D_2 at later exposures.

The vitamin D_2 content was much lower in cultivated mushrooms exposed to sunlight of 200 mJ cm^{-2} UV-B or in wild mushrooms samples compared to mushrooms exposed to supplementary UV-B (Table 1). The samples of cultivated mushrooms without exposure to UV-B or of miscellaneous origin (strains and producers) were found to have the lowest content of vitamin D_2. To our knowledge, the vitamin D_2 content of 1.5 µg 100 g^{-1} is the first report on wild *Agaricus* sp., while higher contents have been reported for other wild grown species such as *Cantharellus cibarius* and *C. tubaeformis* (10–13 and 21–30 µg 100 g^{-1}, respectively) as well as for *Boletus edulis* (3–59 µg 100 g^{-1}) (1, 2). The low vitamin D_2 contents of wild *Agaricus* sp. and cultivated *A. bisporus* exposed to 200 mJ cm^{-2} UV-B in sunlight were surprising considering the results of other reports and the present study of cultivated *A. bisporus* forming high amounts of vitamin D_2 when exposed to supplementary UV-B (6, 4, 8). Even though *A. bisporus* does not produce as high levels of vitamin D_2 after UV exposure as wild *C. tubaeformis* (2) and cultivated *Pleurotus ostreatus* (5). The difference between the effect of UV-B from sunlight and from the supplementary UV-B unit may have been caused by differences in the distribution of wavelengths within the range of UV-B that are integrated in the measurement of the UV-B sensor. The tubes of the UV-B unit emit some

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Table 1. The effect of source of UV-B and timing of exposure in relation to harvest on vitamin D_2 in wild and cultivated *Agaricus* mushrooms

Origin	UV-B source	Relative to harvest	UV-B dose mJ cm^{-2}	Dry matter content g $100\ g^{-1}$ Fresh weight (FW)		Vitamin D_2 concentration µg $100\ g^{-1}$ FW		
Cultivated	UV-B tubes	Prior	200	8.4	e[§]	24.1	(22.9–25.4) [†]	b
Cultivated	UV-B tubes	Post, cooled	200	8.6	e	32.4	(27.2–38.7)	a
Wild	Sunlight	Prior		12.3	ab	1.5	(0.7–2.3)	c
Cultivated	Sunlight	Post	200	9.4	de	0.9	(0.8–1.1)	c
Cultivated	–	–	0	10.4	cd	0.3	(0.04–0.9)	d
Cultivated, misc origin	–	–	–	11.5	bc	0.2	(0.1–0.4) [‡]	d

[§]Within columns means followed by different letters are significantly different. Means followed by the same letter are not ($P<0.05$; $n=4$); [†]Range; [‡]Samples taken from other flush.

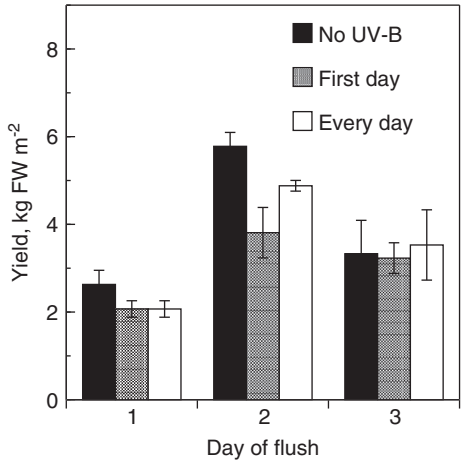

Fig. 3. The yield of mushrooms obtained during the three days of Experiment A after (a) no exposure to UV-B, exposure to supplementary UV-B of 200 mJ cm^{-2}, (b) the first day and (c) repeated every day. Error bars indicate standard errors ($n=4$). FW = fresh weight.

radiation of wavelengths shorter than 290 nm (Fig. 1), which is the part of the UV-B range that is almost absent in sunlight after absorption in the earth's atmosphere (9). This short-waved UV-B is close to the UV-C range that has, however, been found to be less effective than UV-B in the conversion of ergosterol to vitamin D_2 (5). Reports on effects of sunlight on vitamin D_2 in mushrooms are scarce. *Lentinus edodes* was found to have higher vitamin D_2 content when harvested on sunny as compared to cloudy days (10 as reported in (4)). The production of mushroom usually takes place in dark environment. The UV-exposure is similar to the exposure of wild mushrooms that is not reported to cause toxic elements in mushrooms.

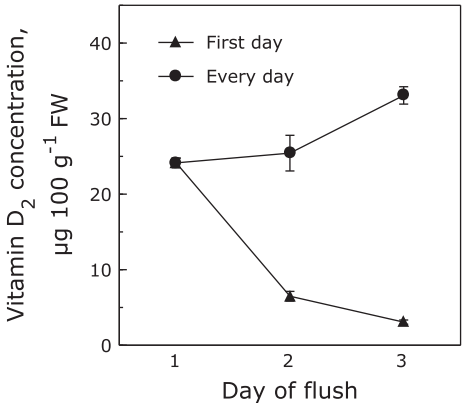

Fig. 4. The vitamin D_2 concentration in mushrooms found during the three days of Experiment A after exposure to supplementary UV-B of 200 mJ cm^{-2} (a) the first day, and (b) repeated every day. Error bars indicate standard errors ($n=4$). FW = fresh weight.

Table 2. Recovery of vitamin D_2 calculated on basis of production area for three days of experiment A after one exposure to supplementary UV-B of 200 mJ cm^{-2} the first day

Day of experiment	Cumulated harvested mushrooms	Remaining mushrooms for next day[a]	Sum	Recovery of vitamin D_2[b] %
	μg vitamin D_2 m^{-2} shelf area			
1	499[c]	435[c]	934	–
2	746	98	844	90
3	845	31	876	94

[a]Based on the biomass of small remaining mushrooms and the assumption that their vitamin D_2 content equalled that measured in large harvested mushrooms.
[b]Based on the sum of vitamin D_2 at Day 1 as 100%.
[c]$n = 4$.

The level of vitamin D_2 content in wild and cultivated mushrooms with or without exposure to sunlight (Table 1) is comparable to that of, for example, 0.4–0.7 μg of vitamin D_3 in 100 g^{-1} of pork and beef meat and 0.2–0.4 μg of vitamin D_3 in 100 g^{-1} of a variety of cheeses (www.foodcomp.dk).

Commercial growers may consider using UV-B during the growth phase for vitamin D_2 formation in white button mushrooms. In this case, the need for prolonged exposure time is less critical as treatment times do not have to be confined to the time available during the post-harvest processing on a conveyor belt. Instead, an automatic moving UV-B unit can be constructed, for example, for overnight exposure of mushrooms on the growing shelves just prior to harvest. With the high intensities used in the present study, doses of 200 mJ cm^{-2} could be obtained with an exposure time of <2 min and resulting in vitamin D_2 contents of around 24 μg 100 g^{-1}. At this vitamin D_2 level, approximately 35 g of mushroom is equivalent to the recommended daily intake of 7.5 μg vitamin D_2 for adults in Denmark. The upper safe limit for daily intake of 50 μg vitamin D_2 should be taken into consideration (11). Mushrooms with a vitamin D_2 content of 24 μg 100 g^{-1} would contribute to 26% of the recommended daily intake of 7.5 μg based on the average intake of mushrooms by adults of 3 kg yr^{-1} in 2006 in Denmark (12). Therefore, UV-B-enhanced vitamin D_2 levels in mushrooms provide a potentially important food source of vitamin D.

Conclusion

The close to linear relationship between UV-B dose and formation of vitamin D_2 at exposure just prior to harvest makes it possible to produce mushrooms with a well-defined content of vitamin D_2. Therefore, mushroom products will be a rich source for increased intake of vitamin D_2 in food. Due to the dilution of vitamin D_2 by the fast growth of the mushrooms, UV-B exposure needs to be repeated daily during the harvest flush to maintain a high content of vitamin D_2 in the end product. Supplementary UV-B from tubes is a more effective source of UV-B for the formation of vitamin D_2 in mushrooms as compared to natural sunlight that gave a low content of vitamin D_2 equivalent to that found in wild *Agaricus* sp. mushrooms.

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Conflict of interest and funding

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